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Metabolic and Nutritional Factors Influencing the Development of Competence for Transfection of *Bacillus subtilis*

KENNETH F. BOTT AND GARY A. WILSON

Department of Microbiology, University of Chicago, Chicago, Illinois 60637

INTRODUCTION

Despite the remarkable diversity among the bacterial species which can be transformed by purified deoxyribonucleic acid (DNA), there appears to be considerable uniformity in the mechanism of the transformation process. The steps of the transformation process have been known for some time and have been considered in several reviews (13, 15, 19), but for clarity the common features are briefly summarized below. (i) A reversible association of the DNA with recipient cells occurs. (ii) Irreversible binding and nonspecific incorporation of high molecular weight DNA, achieved by means of an energy-consuming process, result in a state of deoxyribonuclease insensitivity. The recipient cells have a unique and transient physiological capability, referred to as competence, which enables them to carry out this process. This capacity is itself controlled by at least one genetic locus. (iii) A period of latency follows during which nonhomologous DNA (and much homologous DNA) is degraded to acid soluble components. (iv) Single-stranded portions of donor molecules which have survived degradation are integrated to replace approximately homologous sections of recipient DNA. This process resembles repair synthesis of DNA and not extensive DNA synthesis, since the newly integrated region is probably not much greater than several thousand nucleotides in extent (2). Under normal physiological conditions, the competent cells cannot incorporate single-stranded DNA directly; however, by manipulating the recipient culture during certain stages of growth, or stressing the physical boundaries of the cell, various species can be induced to take up DNA under conditions where it would otherwise be excluded (4, 5, 7, 12). Finally, (v) segregation and expression of the newly incorporated genetic information occurs, leading to the production of genetically stable progeny bacterial cells that are phenotypically distinguishable from the original recipient population.

Special note should be made of the fact that, under the normal physiological conditions which are required for competence development, none of the transformable species has been shown to be capable of conjugation. This leads to the speculation that transformation may represent the normal means of genetic exchange in these species.

Spizizen and co-workers made a very significant contribution to the study of microbial genetics when they identified and characterized the transformation system in *Bacillus subtilis* (1, 24, 25). This system has numerous advantages over other transformation systems. Important among these are the ability of *B. subtilis* to grow and develop competence in a simply defined glucose-salts growth medium, its nonpathogenicity, and its well-characterized built-in system of cellular differentiation—the process of endospore formation. However, the relative inefficiency of the *B. subtilis* system is one serious disadvantage for many genetic studies. In the system which Spizizen characterized, only 1 to 4% of the recipient population is actually in a state of competence when this characteristic is being maximally expressed. This must be compared with levels of competence which exceed 90% in other species.

The following considerations led us to undertake a study of the physiology of competence development in *B. subtilis*. (i) The expression of competence is a peculiar prerequisite for DNA uptake in all transformable systems known, yet there appears to be little or no uniformity in the characteristics of the formation of this stage among the widely diverse transformable species. A better understanding of the physiology of the cells involved might bring to light some unifying characteristics. (ii) In recent years, considerable attention has been focused on the functioning of *B. subtilis* genetic material as it relates to the

morphological transition which initiates spore formation. The structural aspects of this system have been extensively characterized (9), so the species seems especially well suited for studies of cellular differentiation at the unicellular level. The development of competence represents still another form of differentiation in this species, since it reflects a selective or differential functioning of the genes involved in bringing a cell into the state of competence. (iii) Most of the other transformation systems are characterized by a more efficient expression of competence, although, like the presently used system for *B. subtilis*, they all require specialized growth conditions for expression of this transitory stage of maximal efficiency.

It occurred to us that if we systematically looked at the cultural conditions required for competence development in *B. subtilis*, we might be able to define improved conditions, for this expression. In the process, we would contribute significantly towards identifying conditions for physiological expression of this type of differentiation, we would be in a better position to relate it to the more well-known differentiation of sporulation, and we would provide the geneticists with a more useful tool for their studies. As an added benefit, we hoped that these studies would clarify the reasons why some other species of *Bacillus* are not capable of developing competence. Being a bit speculative, we also hoped that the studies would enable us to identify the favorable conditions in such a manner that other *Bacillus* species could be made competent by use of a similar technique.

In the course of our studies to date, we have made reasonable progress toward these goals, having identified more specifically the physiological conditions which prevail during the expression of maximal competence. We can now routinely obtain populations composed of between 5 and 10% competent cells. However, the very fact that we have managed to increase the level significantly by refining the cultural conditions has reinforced our belief that there does exist a specific set of conditions which, when found, will enable us to produce from any genetically suited strain a population of cells which is nearly, if not entirely, composed of competent cells.

The test system used in our studies has evolved from attempts to simplify the actual transformation process in such a manner that we can direct our attention specifically toward the aspect of competence. We have capitalized on the fact that in all transformable systems DNA uptake is nonspecific; i.e., there is no discrimination for a particular species of DNA as long as the DNA is in the native state and is of sufficient molecular weight. We used the DNA from bacteriophage ϕ29 because every competent cell which has taken up this DNA will be expressed as an infective center when tested in the presence of excess noninfected bacteria. This process is referred to as transfection. We assume that it bypasses the necessity for recombination with the host genome as well as the selection for transformants with a specific phenotype; the latter would of necessity reveal only a fraction of the total transformed cells.

Phage ϕ29 was chosen specifically because its DNA is relatively small (10^7 daltons), and therefore our chances of extracting and purifying the genome intact from the phage particles are increased. This phage was also chosen because it was shown that its production of transfectants is directly proportional to the concentration of DNA added (14; B. E. Reilly, Ph.D. Thesis, Western Reserve Univ., Cleveland, Ohio, 1965).

We recognize that by using such a system we are actually defining a competent cell as one which can take up native phage DNA and carry out the process of phage synthesis with an efficiency great enough to liberate at least one viable phage particle. This particle must infect the indicator strain for the original cell to be recorded as an infectious center.

There are a few inadequacies in the system, but at this stage of our knowledge they do not appear to influence the interpretation of our results. As a precaution, each major improvement in the conditions for transfection is checked by evaluating its effects on the transformation of bacterial markers. Very few differences have yet been noted. Several of the recognized faults will be mentioned, however, since they may be helpful to other investigators who wish to evaluate the results for other purposes.

One possible limitation is that competent cells, which are known to be "biosynthetically latent" in several physiological roles (10, 11, 19), are required by the transfection assay to become host for bacteriophage synthesis during a portion of their growth cycle (and in a growth medium) which is not usually associated with maximal efficiency for this process. In addition, ϕ29 is not a virulent bacteriophage for the *B. subtilis* strain (168 or Mu8u5ul) in which competence is being assayed. The plaques produced by phage (or DNA) infection are very turbid and are not easily scored on this host, making it more beneficial to use another (nontransformable) strain for an indicator. This strain originally designated *B. subtilis* H by Reilly and Spizizen (14) has recently been shown to be *B. amyloliquefaciens* (21). Furthermore, although the number of transfect-

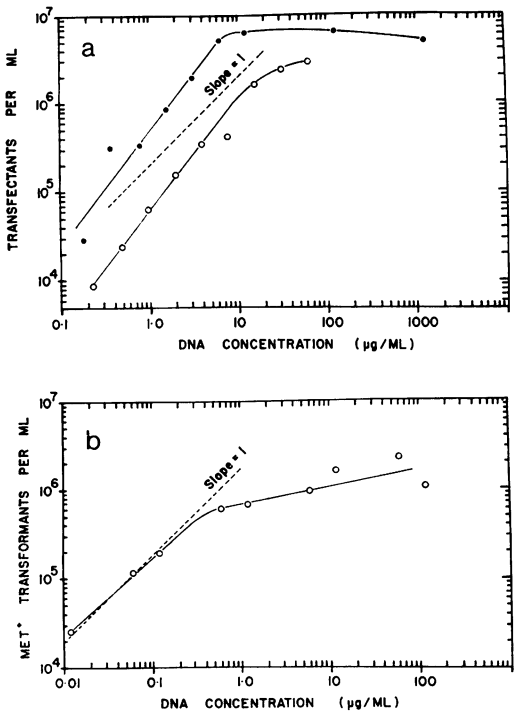

FIG. 1. *Saturation curves for bacteriophage φ29 DNA and bacterial DNA. (a) DNA isolated from phage which was pelleted by centrifugation at 33,000 × g for 120 min (●). Bacterial debris had been removed from the crude lysate by low-speed centrifugation. DNA isolated from phage particles which were banded in cesium chloride after treatment with lysozyme and deoxyribonuclease (○). Two different recipient populations of bacterial strain Mu8u5u1 were used, which explains the low plateau in the lower curve. In each, the time of DNA exposure was 40 min, followed by 10-min treatment with deoxyribonuclease (0.1 ml, 100 μg). (b) DNA isolated from bacterial strain 168T+. The recipient strain was strain Mu8u5u1. Time of exposure to DNA was 40 min. Transformants were scored on minimal medium lacking methionine. All procedures were as outlined previously (3, 22).*

ants is *almost* proportional to the concentration of DNA added, it is not exact. Our experiments, and recalculations of the data of Reilly (Ph.D. Thesis, Western Reserve Univ., Cleveland, Ohio, 1965), indicate that, instead of illustrating a slope of one, the slope is actually 1.13 (Fig. 1a), a fact which suggests that perhaps some slight interaction between phage molecules is necessary on the average for the production of each infectious center. In addition, as shown by Reilly, about 10,000 equivalents of phage DNA are required for the production of each infectious center. Another difference between this transfection system and the transformation system is that the level of DNA required to saturate a population of competent cells is not 1.0 μg of DNA per ml as with bacterial DNA (Fig. 1b) but 8 to 12 μg/ml (depending on whether the phage was extracted from CsCl banded phage). This discrepancy may reflect the requirement of 10,000 phage equivalent for the expression of each plaque, but it leads one to speculate that all of the DNA in the preparations is not competing for competent cell sites. Sedimentation analyses of φ29 DNA preparations suggest that the DNA molecules are homogeneous and of uniform size.

One further point which must be recognized is that our studies to date have examined the effects of various substances on the combined processes of growth and transfection or transformation. As a result, a substance considered to stimulate transformation in our system must support both development of competence and efficient expression of the incorporated DNA, while inhibitory substances might actually have their effect on either of these. Most other studies to date have been concerned with conditions which favor the expression of competence which was developed under a standard regimen.

EXPERIMENTAL OBSERVATIONS

Figure 2 serves to illustrate our basic system and helps to review briefly the characteristics which have been reported (3, 22). We shall also summarize some unpublished findings which contribute to our understanding of what makes competent cells competent. The experimental protocol used follows the methods already published (3, 22), and any modifications of these procedures will be designated.

We have shown that, when cells are monitored spectrophotometrically during growth in a chemically defined medium, competence reaches a peak of activity 2 to 3 hr after T_0 (the cessation of exponential growth), regardless of the duration or extent of growth during the exponential phase (3). A slight but consistent reduction in viability usually coincides with the point at which competence is maximal. This knowledge permits a refinement of the procedure used by Spizizen and co-workers, since it places the peak of competence at a definite stage of growth and eliminates the necessity for transferring the culture to a second medium or otherwise manipulating the culture during the competence regimen. Furthermore, we have found that casein hydrolysate which was formerly a component of this system, actually contains several amino acids which inhibit the development of maximal levels of competence (22). We have replaced the casein hydrolysate with a mixture of nine amino acids which enhance the development of competence and have

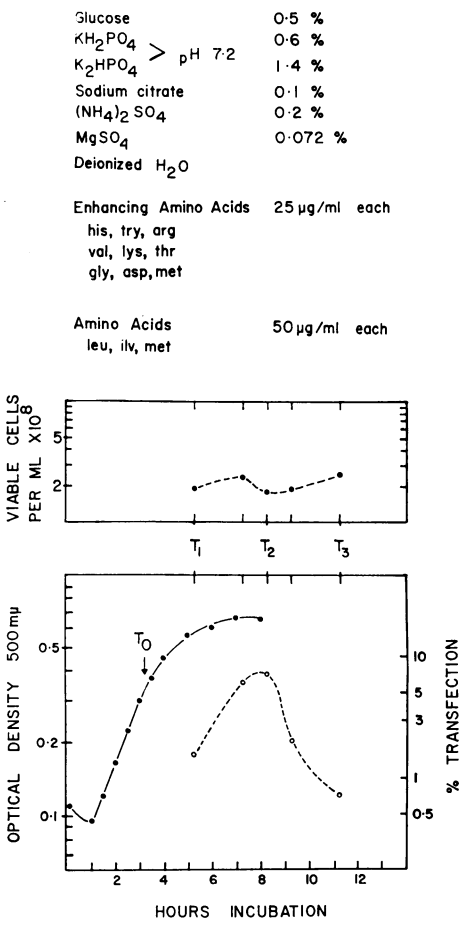

FIG. 2. *Synthetic medium for growth and transfection (or transformation); expression of peak competence. Saturating levels of φ29 DNA (15 to 25 µg) in 0.1 ml were added to 0.9 ml of bacterial cells, and the mixtures were incubated at 37 C for 40 min with rapid agitation. After a 10-min treatment with deoxyribonuclease (100 µg in 0.1 ml), the mixture was diluted and plated as in reference 3, with B. subtilis strain H as indicator in soft agar. B. subtilis strain Mu8u5u1 was the recipient strain in all experiments unless otherwise noted.*

eliminated those which have no effect or which are inhibitory.

These refinements have provided us with a completely defined synthetic medium which produces competent cells capable of transfection at a frequency of 5 to 10%. On several occasions we have obtained levels as high as 15 to 18%.

It is important to stress that the single most influential criterion for obtaining good reproducibility with this system is the purity of the water used to prepare the medium. As will be mentioned later, several inorganic cations drastically reduce the levels of competence obtained. Anagnostopoulos and Spizizen (1) believed that one of the major assets of the casein hydrolysate in their system was its ability to act as a chelating agent for undetermined ions. The purity of the water becomes essential if one remembers that some of these chelating agents have been removed from our system. We have found that demineralized water freed from organic matter by prefiltration through a charcoal filter is superior to doubly glass-distilled water.

Our results indicate that, at the time when exponential growth ceases, less than half of the glucose has been consumed. There is no effect on the growth curve or development of competence if one-half or twice the normal amount glucose is used in the medium. Glucostat (Worthington Biochemical Corp., Freehold, N.J.) analyses of culture supernatant fluids clearly indicate that less than 50% of the initial glucose has been used by the time peak competence is observed.

In other experiments, the glucose in the medium was replaced with 0.5% of glycerol, sucrose, sodium succinate, or sodium acetate (Table 1). As expected, the rates of exponential growth varied considerably depending on the carbon source used, but very dramatic effects on the development of competence were noted. Sucrose supports a reasonable level of competence development, although maximal competence appears at T_3. The gradual rise to maximal efficiency that typifies the glucose-grown cultures may be modified in this medium to more closely resemble a bimodal peak of competence having a maximal value in the second peak; at this point, the significance of these observations is not clear. Glycerol is a poorer source of carbon for competence development; it produced populations with only 5% of the competence that developed in glucose-grown cultures. Succinate and acetate are definitely inhibitory, since they produced competent cells at a frequency 2.5 to 3 orders of magnitude lower than glucose.

The results of our experiments with glycerol and sucrose prompted us to test the effects of using sucrose instead of glycerol for the preservation of competent cultures in the frozen state, since some of the effects observed may be due to an inhibitory activity at the time of contact with DNA as well as to inhibition of competence development. Our results indicate that recovery of competent cells is *not* more efficient upon thawing (after 1-week storage at −60 C) when an 80% sucrose solution is used in place of glycerol (at a concentration of 15%) as a preservative. Young and Spizizen (25) reported that no adverse effects on transformation were detected when competent

TABLE 1. *Effects of various carbon sources on development of competence*[a]

Carbon source	Frequency of transfection at indicated times after exponential growth				
	1 hr	2 hr	2.5 hr	3 hr	4 hr
	%	%	%	%	%
Glucose...	1.5	6.0	7.1	2.0	0.68
Glycerol..	0.25	0.34	0.29	0.31	0.38
Sucrose...	2.0	1.7	0.93	4.5	1.13
Succinate.	0.0034	0.002	0.008	0.007	0.0014
Acetate...	<0.001	0.002	<0.001	<0.001	<0.001

[a] Growth medium prepared as indicated in Fig. 2 was modified by replacing the glucose with 0.5% of the carbon source indicated. Growth was monitored spectrophotometrically until the end of exponential growth (T_0) could be determined. At intervals after this point, 0.9 ml of the culture was transfected as described in the legend to Fig. 2 and in reference 3. Viability was determined at the time of DNA addition by plating 0.1 ml of a 10^5 dilution on tryptose blood agar base plates (Difco). Transfection frequency was determined by dividing the number of infectious centers per milliliter by the viability. This value is multiplied by 100 to give per cent. The values for glucose represent the average of eight experiments in which this was used as the control medium for tests of each carbon source. The values for other carbon sources represent the average of two experiments in which that medium was tested.

cells were incubated in media containing pyruvate or lactate as the carbon source. Together, these results suggest that there may be a differential effect between competence development and expression under the same experimental conditions.

In the next series of experiments, we examined the effects of *p*H on expression of competence. In the first of these experiments, cultures were grown in the conventional glucose medium to $T_{2.5}$, and then 5-ml samples were removed and adjusted to a different *p*H with either 1 N HCl or 1 N NaOH. Viability was determined for each adjusted sample, and the frequency of transfection was noted after exposure to phage DNA. As shown in Fig. 3, a sharp peak of competence expression occurred at *p*H 6.7. These results confirm the observations of Young and Spizizen (25). In subsequent experiments, the phosphate component of the medium was altered so that its buffering capacity would be at *p*H 6.0, 6.7, or 7.5. Cultures grown in these media were compared in the same experiment with cultures grown in the conventional medium which is buffered at *p*H 7.2. The peak of competence was highest in the *p*H 6.7 medium, although not six times higher as had been observed in the experiments shown in

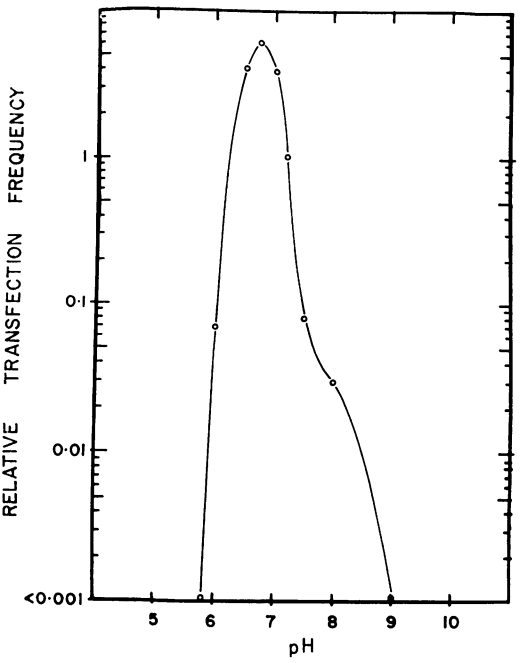

FIG. 3. *Effects of pH on competence expression.*

Fig. 3. The value was 2.5 and 3.0 times greater in two experiments at *p*H 6.7. In more recent experiments, we have employed this altered buffer (0.76% KH_2PO_4, 1.24% K_2HPO_4) so that competence will develop at *p*H 6.7. Under the normal growth conditions in these media, there is no notable change in the *p*H during the course of growth and competence development.

Sodium citrate was slightly stimulatory to competence development when the concentration was halved, but the transfection frequency was so close to that of the control cultures that it has not yet been established that the lower level is significant.

Another increase in frequency of competence can be attained by supplementing the medium with additional nitrogen, either as $(NH_4)_2SO_4$ or as KNO_3 (Fig. 4). At equivalent concentrations, these two nitrogen sources were interchangeable for growth and competence development. Higher levels of these nitrogen sources were accompanied by a slight increase in cell viability, suggesting that nitrogen may stimulate more growth in this medium. A shift of the peak of competence to a slightly later point was noted when concentrations were raised to 0.6%. If $(NH_4)_2SO_4$ was supplemented by adding 0.2% NH_4HCO_3, competence reached its peak at T_3 or later and did not attain levels comparable to that of the unaltered control.

The final component, magnesium, is known to

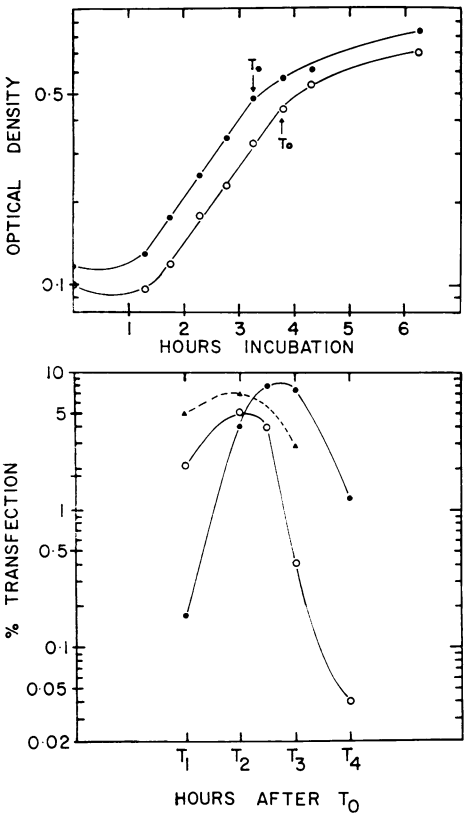

FIG. 4. *Development of competence in the presence of altered nitrogen component. (top) Growth of control culture (○) in medium shown in Fig. 2 and that of three test cultures. All cultures were inoculated with samples from the same overnight suspension. Test cultures contained 0.4% $(NH_4)_2SO_4$, 0.6% $(NH_4)_2SO_4$ or 0.6% KNO_3 as the nitrogen source. One curve is illustrated (●) since growth of the three cultures was identical. (bottom) Transfection frequency (as %) when the control (○), 0.4% $(NH_4)_2SO_4$ (▲), and 0.6% $(NH_4)_2SO_4$ or 0.6% KNO_3 (●) were transfected at hourly intervals after the cessation of exponential growth. The curves for 0.6% KNO_3 and 0.6% $(NH_4)_2SO_4$ were identical and are indicated by a single curve.*

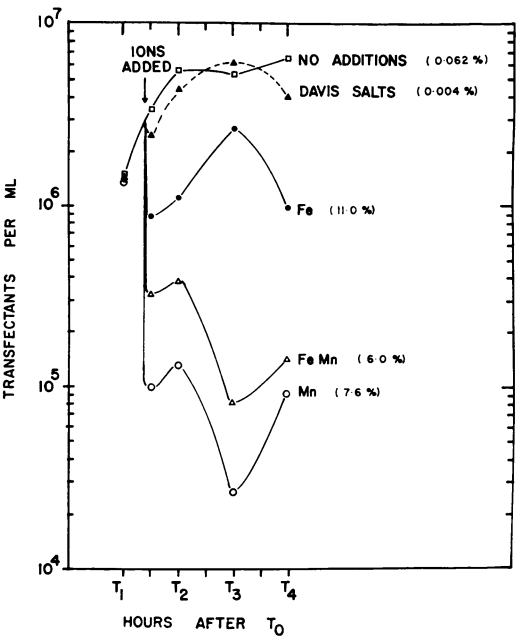

FIG. 5. *Effect of Mn and Fe on competence development. Five flasks were inoculated with strain U1 and growth was followed in the usual manner at 30 C. All cultures reach T_0 simultaneously and were transfected 1 hr later (T_1). Also at this time, a 0.1-ml sample was removed and diluted 100-fold. A 1-ml amount of this dilution was heat-shocked for 10 min at 80 C in a tube capped to prevent evaporation, and the remainder was used to determine total viability. After heating, the 1-ml sample was plunged into an ice bath, and viability was determined again. The survivors reflect the number of mature spores, and from these two values the per cent sporulation was calculated. Five minutes before $T_{1.5}$, 0.65 ml of $MnCl_2$ was added to one of the flasks (○) to give a final concentration of 10 μg/ml. Another flask received 0.65 ml of $FeCl_3$ (●) to give 50 μg/ml; the third flask received 0.65 ml containing both Mn and Fe in the above concentrations (△). Controls included the fourth flask which received 0.65 ml of minimal salts (▲), and the fifth flask which was untreated (□). At $T_{1.5}$, T_2, and hourly intervals thereafter, all five cultures were transfected. Samples from each culture were heat-shocked at T_3, T_5, T_{24}, and T_{48}. The percentage increase in sporulation frequencies after 48 hr is given parenthetically for each culture.*

be important at this relatively high level for maximal expression of competence (3). Lower levels do not materially affect growth but do limit competence development. Increasing the concentration to four times this amount (0.29%) has no apparent effect on growth or competence, but does lead to precipitation in the medium. One possible effect of this ion may be a role in stabilizing the cell population by virtue of its ability to inhibit the proteolytic enzymes present in *Bacillus* (J. Mandelstam, W. M. Waites, and S. C. Warren, Intern. Congr. Biochem., 7th, Tokyo, 1967). This component deserves a more intensive investigation, since our preliminary results have suggested that altered levels may influence a differential expression of transfection and transformation.

When all of these beneficial modifications are incorporated into a single growth medium, reproducibly high levels of competence do result, but we have not yet achieved a level high enough to convince us that a majority of the cells are com-

petent. Routine transfection assays are close to 10%; as suggested earlier (22), this may be an underestimate which is inherent in this transfection assay. Our current efforts are directed towards improving this technique, as well as defining more clearly the nature of competence.

Several lines of evidence lead us to believe that competence in *B. subtilis* is actually brought about by a metabolic blockage. This could be a block in a pathway which would normally lead to endospore formation. Such a theory was suggested by Spizizen and co-workers (17–19). It is supported by: (i) the inability of several asporogenous mutants to develop competence (18), (ii) the linked transformation between competence and some sporulation genes (17, 18), (iii) the transitory nature of competence expression (3), (iv) the biosynthetic latency of competent cells (11), (v) resistance of the competent fraction to penicillin (10), and (vi) the manner in which iron and manganese immediately reverse the expression of competence (Figure 5).

The medium used in growing *B. subtilis* to competence is notably deficient in two essential ions required for the sporulation process—iron and manganese (26); neither is essential for vegetative growth of *B. subtilis* in more than trace amounts. If either of these ions is added to the regular growth medium, competence does not develop; either or both of these will assure that sporulation will occur at significant levels. Furthermore, if these ions are added singly or together to a normal population after competence has begun to develop, they effectively inhibit competence expression within 5 min (Fig. 5). One possible interpretation of these results is that cells reach a critical step while initiating the early stages of sporulation at or near a point where these ions are needed for unimpaired development of the process. Perhaps presporulation enzymes have been induced which need one or both of these ions to function. Inability of this process to be completed, or reversed (since cells are in stationary phase), could lead to altered permeability properties of the cell, especially if the cell wall was weakened at the site of a prespore septum (23). According to this view, a substance could inhibit competence by reversing the sporulation block or strengthening the cell wall, as well as by more general methods which interfere with cell metabolism.

The physiological conditions at the time of competence expression (8, 11) are consistent with our knowledge of what takes place during presporulation metabolism (9), and the amino acids which were shown to inhibit competence development are those known to be major components of the cell wall of *B. subtilis*. (22). This theory would suggest that the asporogenous mutants which develop full competence (15) might be blocked at a later stage in the sporulation process.

If a blockage does exist, this point must occur before the cell has become irreversibly committed to spore synthesis, since transformed cultures do not first pass through the spore phase. This point

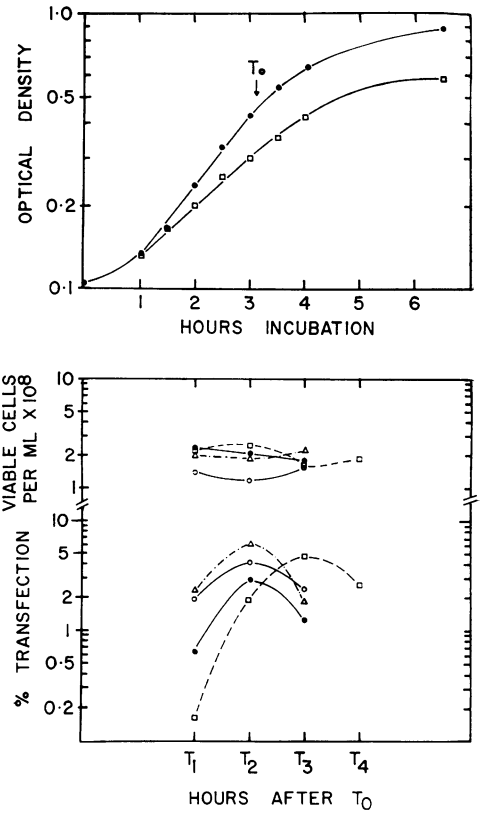

FIG. 6. *Effects of α-picolinic acid and sodium malonate on competence development. (top) Five 1-liter flasks containing 100 ml of growth medium (as in Fig. 2) were inoculated from the same overnight suspension of strain Mu8u5u1, and growth at 37 C was monitored spectrophotometrically. The four flasks containing no additions, 50 μg of α-picolinic acid per ml, 150 μg of α-picolinic acid per ml, and 1.6 mg of sodium malonate per ml added at the beginning of growth gave an identical growth response and are indicated by a single curve (●). The flask in which 250 μg of α-picolinic acid per ml was added at the beginning of growth (□) grew slightly slower. (bottom) Transfection frequency (as %) and viability obtained when samples from each of the five flasks were transfected at hourly intervals after the cessation of exponential growth. Symbols: ●, control; ○, 1.6 mg of sodium malonate per ml; △, 50 and 150 μg of α-picolinic acid per ml (the two cultures were identical); □, 250 μg of α-picolinic acid per ml, which caused the peak to appear 1 hr later.*

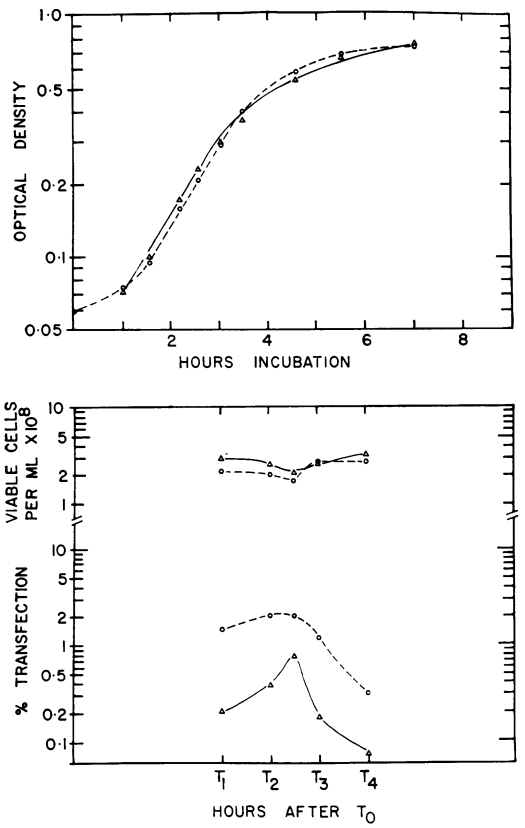

FIG. 7. *Effect of sodium fluoroacetate on growth and competence. (top) Two 1-liter flasks were inoculated with 100 ml of growth medium (as in Fig. 2) from the same overnight suspension of strain Mu8u5u1. After 1 hr of incubation at 37 C, fluoroacetate was added to one flask (△) to a final concentration of 500 μg/ml. The other flask was unaltered and served as the control (○). Growth proceeded normally in both cultures. (bottom) Viability and transfection of samples from the same two flasks at hourly intervals after cessation of exponential growth.*

is further illustrated by the experiments illustrated in Fig. 6 and 7. Populations can be grown normally and achieve competence in the presence of concentrations of α-picolinic acid, sodium malonate, or sodium fluoroacetate which have been shown to inhibit sporulation. All three of these chemicals are known to inhibit sporulation but not vegetative growth of various *Bacillus* species (9). All are proposed to inhibit sporulation by competitively inhibiting the tricarboxylic acid cycle, which is essential for spore synthesis. Induction of tricarboxylic acid cycle enzymes has been shown to be a necessary prerequisite for the early biochemical changes leading to spore synthesis (20).

Figure 6 indicates that α-picolinic acid at concentrations as high as 150 μg/ml and sodium malonate at 1.6 mg/ml alter neither growth nor competence development. α-Picolinic acid at a concentration of 250 μg/ml did slightly inhibit the growth rate and the appearance of maximal competence, but a slight stimulatory effect was noted in expression of competence at the peak. Spizizen et al. have indicated an inhibitory effect of α-picolinic acid on competence (19), but the results cannot be compared since the conditions of their experiments were not specified.

Fluoroacetate at 500 μg/ml (Fig. 7) slightly depressed the maximal level of competence development but did not affect its appearance at the normal time. No dramatic effect directed toward competence development could be shown in these experiments.

Schaeffer et al. (16) have proposed that sporulation can be induced in *B. subtilis* by a lack of either carbon or nitrogen source in the growth medium. In addition, Hansen and Cox (6) have shown that certain of the essential enzyme activities associated with spore synthesis are subject to glucose repression. One reason for the inefficient development of competence in *B. subtilis* may be that essential enzymes in the sporulation process which would normally precede this block are not efficiently induced owing to the presence of excess glucose in the competence medium. Extra ammonium sulfate or KNO_3 may cause enhancement by permitting a population to utilize more glucose before stationary conditions begin, thus relieving some of the glucose repression.

Experiments are in progress to characterize further the state of competence and to improve the transfection assay system with the hope that the conditions for competence expression can be maximized and accurately determined in a larger portion of the population.

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